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FAUNISTIC NOTE

Small flies with high conservation value: first reliable record of *Hyperoscelis veternosa* (Diptera, Canthyloscelidae) from Romania supported by DNA barcode data

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Abstract

Here we present the first reliable record of the rare *Hyperoscelis veternosa* Mamaev and Krivosheina, 1969 from the headwaters area of the Someşul River, Apuseni Natural Park, Romania. Species level identification was made by detailed examination of the single female genitalia, supported by DNA barcode data. The new finding confirms the presence of the species in Romania, whereas a very old, single and doubtful record from the Bucharest area dates back to 1900 needs further confirmation. The presence of the second species of genus *Hyperoscelis* from Romania is of high conservation value, and confirm the persistence of a relict biodiversity of an old-growth forests-spot in the Carpathians.

Keywords

Canthyloscelidae, conservation, DNA barcode data, new record, relict species, the Carpathians, virgin forests.

The family Canthyloscelidae is a small group of Scatopsoidea flies, which has only 12 species worldwide with scattered or "relict-like" distribution in the Holarctic Region (6 species), southern South America (2 spp.) and New Zealand (4 spp.) (Hutson 1977; Martinovsky 1988; Haenni 1997; Oosterbroek 2006; Kjærandsen and Jaschhof 2019). According to the most recent revisions by Amorim (2000) and Ševčík et al. (2016), the family includes the subfamilies Synneurinae (with two genera, *Synneuron* Lundström, 1910 (with 3 spp.) and *Exiliscelis* Hutson, 1977 – with 1 sp.) and Canthyloscelinae (with two genera, *Hyperoscelis* Hardy and Nagatomi, 1960 (with 2 spp.) and *Canthyloscelis* Edwards, 1922 (with 6 spp.). In Europe only three species occur so far, *Hyperoscelis eximia* (Boheman, 1858), *Hyperoscelis veternosa* Mamaev and Krivosheina, 1969, and *Synneuron annulipes* Lundström, 1910 with rather sporadic data (Martinovsky 1972; Haenni and Dufour 1983; Klasa 1991; Mikołajczyk 1993; Schacht 1993).

Members of the family are considered remnants of an ancient fauna restricted to some old growing primeval forests, with rarely collected adults and larvae detected from wet, rooting woods and stumps near waters (Roháček and Ševčík 2009; Marshall 2012).

The first record of Hyperoscelidae from Romania dates back to the beginning of the 20th century, when Mik (1900) recorded the species *Corynoscelis eximia* (Boheman, 1858; valid name: *H. eximia*), based on a single male specimen collected by Kiefer from the surroundings of Bucharest, Comana forest. The specimen was later revised and identified by Hutson (1977) as *H. veternosa* (Ceianu, 1998). The single known locality of the species *S. annulipes* from Romania is Mehadia (Banat region) and were cited by Zilahi-Sebess (1960) without any more exact collecting data. The third species, *H. eximia* were recorded by Ceianu (1998) from the Eastern Carpathians close to the Ukrainean border (Câmpulung Moldovenesc and Sadău) based on one male and one female individuals. Apart from these few records, no further data on this relict family in Romania are known so far.

Material examined. 1 female, Bihor county, Ic Ponor on the road to Padiș, Apuseni Mts., Romania, spruce fir forest in marshy area, 46.631716° N/22.805208° E, 1035 m a.s.l.; 7 May, 2021, Keresztes Lujza leg. The specimen (Fig. 1) was collected by sweeping the fallen roots and stamps from a marshy area near the spring sector of the river Someșul Cald. The specimen, with individual code DICN_SC2a_01, is deposited in the Diptera Collection of the Faculty of Biology and Geology (acronym DCFBG), University Babeș-Bolyai, Cluj-Napoca, Romania. Female terminalia were left overnight in 10% KOH and thereafter one hour in undiluted glacial acetic acid to neutralize and wash out the soap that was created from the soft tissues. Then, the male terminalia were transferred to a larger amount of glycerol in order to wash out the acid, and then to a drop of glycerol on a slide with rounded excavation. The slide was carefully transferred to the compound microscope in order to take the photos. Finally, the genital parts were placed in micro vials containing 50–50% ethanol and glycerol and were pinned under the dry specimen. Photos of the wing were taken

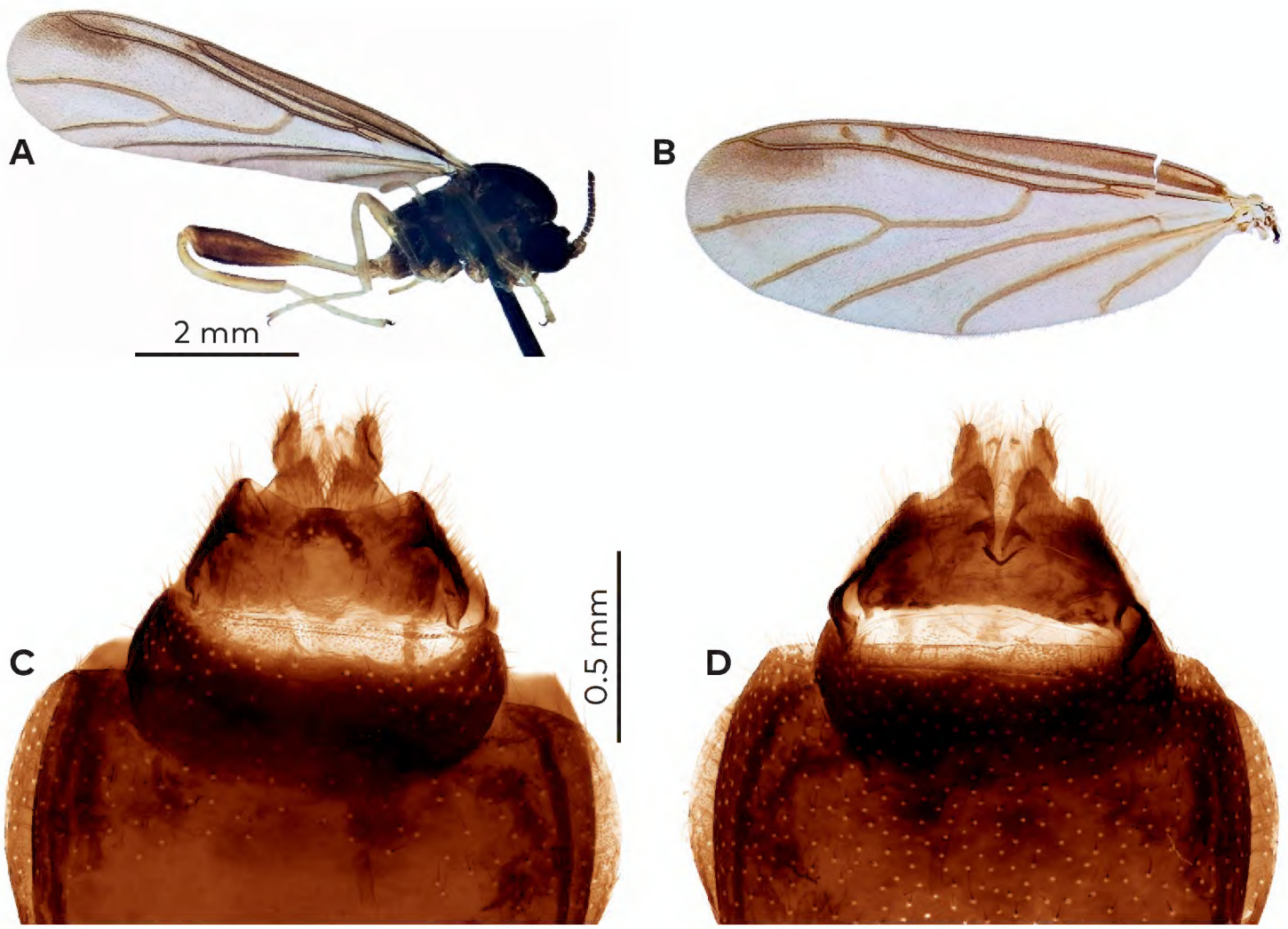

Figure 1. *Hyperoscelis veternosa*, female specimen DICN_SC2a_01. **A** Specimen, lateral view; **B** Right wing of female; **C** Abdomen terminal segments, dorsal view; **D** Abdomen terminal segments, ventral view.

with a stereomicroscope (Olympus SZ51) with a Canon EOS 650D digital camera attached. Photos were taken using a Canon EOS 750D digital camera, attached to the microscope (Olympus CX23), with an LM Digital SLR Adapter (MicroTechLab, Austria). Layer photos were combined using Zerene Stacker software.

Stacking results consist of 10–15 single exposures with the stereomicroscope and 20–50 exposures with the compound microscope.

One leg from the female specimen was sent to the Canadian Centre for DNA barcoding (Guelph, Ontario, Canada), for DNA extraction and bi-directional Sanger sequencing as part of the Romanian Macrozoobethos Barcoding Initiative (ROMAC) in Barcode of Life Data System (BOLD). Publicly available *Hyperoscelis* sequences were downloaded from the BOLD database and a Neighbour-Joining tree was generated using 10,000 bootstrap replicates in Mega X (Kumar et al. 2018).

The single female collected by us is the second record of *H. veternosa* in Romania, after the first record of Mik (1900), under the name *Corynoscelis eximia* Boheman (see Hutson 1977 for discussion). Our data is the first reliable record of the species in Romania, and the first record of the species from the Apuseni National Park, Apuseni Mts. The habitat where the female specimen was collected is typical to *H. veternosa,* and is characterized by a large marshy area near the main course of the Someșul

Cald River, above 1000 m a.s.l. (Fig. 2). The area belongs to the Apuseni National Park, with restricted human activity in an old-growth spruce and fir forest with large amounts of fallen trees and dead wood accumulated as a result of undisturbed, natural forest succession.

The specimen collected by us was DNA-barcoded, and as a result, a 658 bp length sequence (the standard DNA region of the mitochondrial cytochrome oxidase gene *mt*COI) was obtained and assigned to the Barcode Index Number (BIN) in BOLD: ADJ6424. Our DNA sequence was compared with the only two other additional sequences available on public genetic databases. The DNA sequence mined from GenBank (NCBI) (sample ID KT316865) and published by Ševčík et al. (2016) has 99,67% genetic similarity with our genetic dataset, while the second public DNA data mined from BOLD (sample ID NORSC2945-18) has 100% similarity with our data. The *H. veternosa* is the sister species of *H. eximia,* based on standard DNA data in BOLD with BIN number: ADS5898, having 11.89% distance to its nearest neighbour (Fig. 3).

Figure 2. Collecting locality of the specimen represents the reliable record of *Hyperoscelis veternosa* in Romania: Ic Ponor, the Apuseni Mountains, marshy spruce-fir forest along the main course of the Someşul Cald River, at 1035 m a.s.l. (46.631716° N/22.805208° E, 1035 m a.s.l.), leg. Keresztes L.

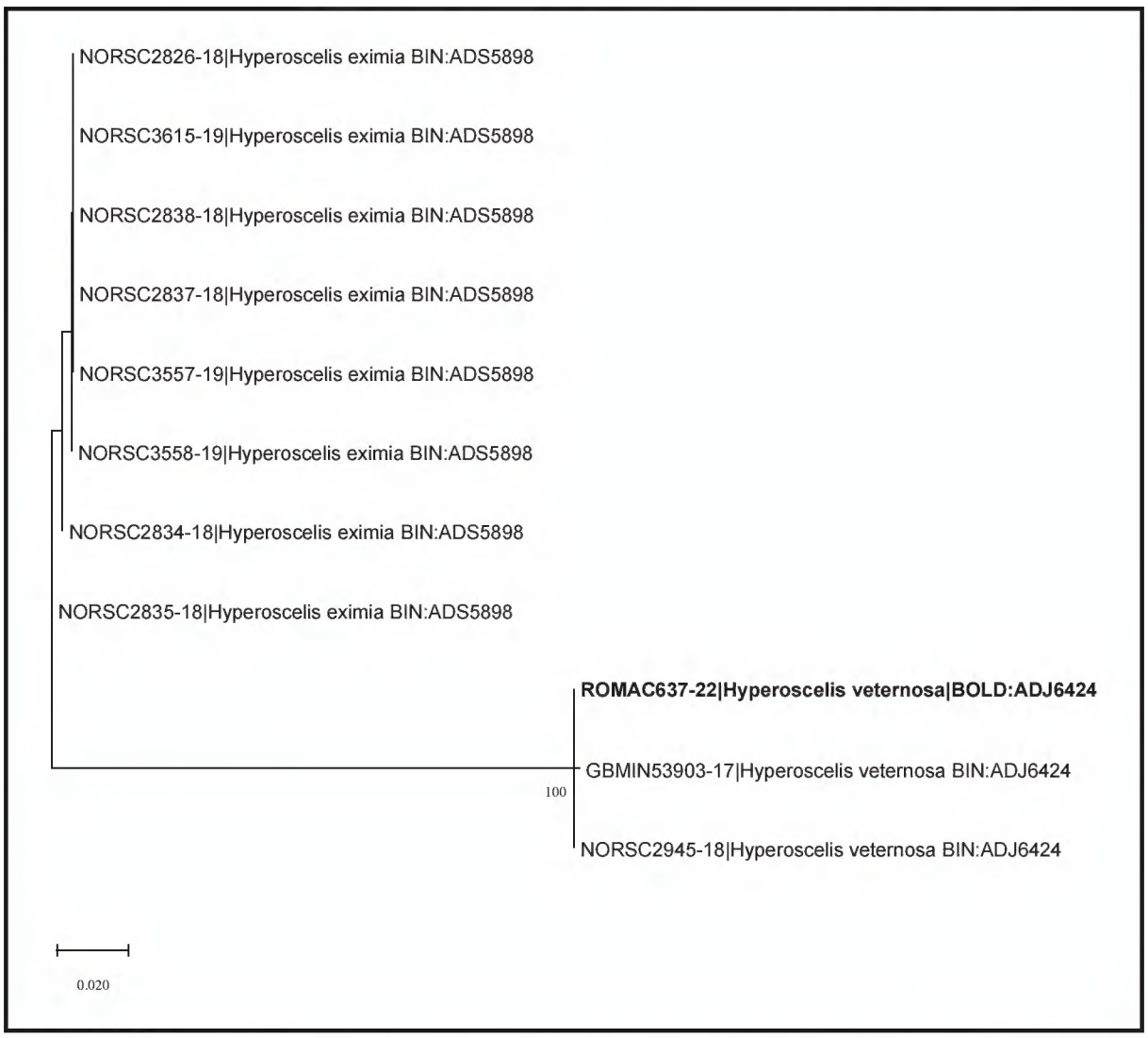

Figure 3. NJ-tree (Kimura-2-distance) with all barcodes available for the European Canthyloscelidae fauna, with their associated BIN numbers. The single specimen collected by us is marked in bold.

Our new data are of great conservation value, as the species has rather sporadic data across Europe and is closely related with primeval forest-type associations, unique ecosystems that are disappearing from most parts of our continent. The presence of the species in the perimeter of the Apuseni National Park protected area suggest the presence of a still highly diverse virgin forests in the Carpathians. The spruce-fir and old-growly beach forests around the headwaters of the Someșul Cald River area needs special conservation efforts and good forest management, as the ongoing global climate change with catastrophic meteorological events affects seriously the area (as it was, for example, in 2017 or in 2022, resulted in massive windfalls of timbers) in combination with the anthropogenic pressure (pasturing, tourism), which will decimate, for sure, these unique ecosystems in the forthcoming decades. The newly discovered *H. veternosa* should be a good indicator species of a still pristine forest ecosystem in this area, therefore we suggest including it, together with the sister species, *H. eximia* in the national red-lists of endangered species with high conservation value.

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